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Retinoic acid-dependent transactivation of major histocompatibility complex class I promoters by the nuclear hormone receptor H-2RIIBP in undifferentiated embryonal carcinoma cells

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ABSTRACT **H-2RIIBP is a member of the nuclear hormone receptor superfamily that binds to the region II enhancer of major histocompatibility complex (MHC) class I genes. The binding occurs through the GG(T/A)CA motif present also in many other genes. The role of H-2RIIBP in developmental regulation of MHC class I genes has been studied in undifferentiated N-Tera2 embryonal carcinoma cells by transient cotransfection of an expressible H-2RIIBP plasmid and a chloramphenicol acetyltransferase reporter gene linked to the MHC class I promoter. Transfection of the expression plasmid led to production of H-2RIIBP transcripts and enhanced MHC class I promoter activity in cells that were treated with retinoic acid but not yet differentiated. Retinoic acid concentrations required for transactivation overlapped with those capable of inducing morphological differentiation and expression of endogenous MHC class I genes in these cells. This enhancement was mediated by region II, as a heterologous thymidine kinase promoter driven by region II also served as a target for H-2RIIBP transactivation. Deletion of the bulk of the DNA-binding domain or the ligand-binding domain of H-2RIIBP, but not of the N-terminal domain, abolished transactivation, indicating that the former two domains are critical for the enhancement. Moreover, H-2RIIBP transactivation exhibited a strict cell-type restriction. As observed in other cell lines, N-Tera2 cells that had undergone differentiation failed to elicit transactivation, suggesting that H-2RIIBP acts in concert with a cofactor expressed in undifferentiated N-Tera2 cells that requires retinoic acid for its function. These results suggest that H-2RIIBP can function as a developmentally specific transcription factor for MHC class I genes.**

Classical steroid and thyroid hormone receptors belong to the nuclear hormone receptor superfamily and regulate hormone-responsive genes. Recently, many additional members of this superfamily have been discovered, whose specific ligands have yet to be identified (ref. 1; reviewed in refs. 2 and 3). These discoveries suggest that this superfamily has diverse functions. We previously isolated a member of this superfamily, called H-2RIIBP, by virtue of its binding to the region II enhancer of major histocompatibility complex (MHC) class I genes (4). Region II is conserved in the murine classical MHC class I genes and elicits a moderate enhancer activity (5). It resides within the conserved upstream regulatory complex mapped from nucleotide (nt) −203 to nt −139 that contains other regulatory elements (Fig. 1*A*; refs. 6–11). Cloned H-2RIIBP and the natural region II binding factor bind the GG(T/A)CA motif which occurs in MHC class I and many other genes, including hormone response genes (B.-Z.L., unpublished work). The GG(T/A)CA motif is also present in phylogenetically distant species and may serve as an evolutionarily conserved hormone response element (12). In accordance, the ancestry of H-2RIIBP can be traced to the *Drosophila* XR2C (or 2C) gene (13, 14). H-2RIIBP is very similar to the human RXRα gene product (15), sharing >85% amino acid identity in the DNA- and ligand-binding domains. Because RXRα can activate a target promoter in the presence of retinoic acid and other retinoids in a manner different from that of retinoic acid receptors, it has been proposed to represent a pathway of retinoid-mediated gene regulation that is independent of the retinoic acid receptors. Since retinoic acid and related molecules act as a developmental morphogen (16) and control morphogenesis in various stages of development (17), H-2RIIBP as well as RXRα may play an important role in development. We have used the human embryonal carcinoma (EC) cell line N-Tera2 (18) to investigate the role of H-2RIIBP in developmental regulation of MHC class I genes. Undifferentiated N-Tera2 and murine EC cells, such as F9 and P19 cells, exhibit properties common with early mammalian embryos (reviewed in ref. 19). For example, both undifferentiated EC cells and early embryos are negative for expression of MHC class I genes (20, 21). Undifferentiated EC cells respond to retinoic acid and induce expression of many genes (22, 23), including MHC class I genes (ref. 24, for F9 cells; J.H.S. and T.N., unpublished data, for N-Tera2 cells). Following changes in gene expression induced by retinoic acid, EC cells differentiate to become a variety of cell types (18, 25). These processes parallel, to some extent, events in developing embryos (26). Thus, gene regulation in EC cells has been studied as a model for mammalian development *in vivo*.

MATERIALS AND METHODS

Plasmid Construction. pRSV-H-2RIIBP was constructed by inserting a 1520-base-pair (bp) *Eco*RI–*Acc* I fragment containing the entire translatable region of the H-2RIIBP cDNA (4) into the *Hin*dIII site of the mammalian expression vector pRSV2 (27). The control pRSV plasmid has a *Bam*HI–*Hin*dIII fragment of ≈1 kilobase (kb) derived from the genomic H-2L^d gene in place of H-2RIIBP, and is not translatable. The three mutants N-del, D-del, and C-del have deletions in the N-terminal, the DNA-binding, and the C-terminal domain, respectively (see Fig. 4*A*). To generate C-del, pRSV-H-2RIIBP was digested with *Sac* I/*Nhe* I and was treated with mung bean nuclease followed by phosphorylation and religation, which resulted in an in-frame deletion of nt 871–1332. N-del and D-del were created by a two-step deletion. The nucleotide sequences of these mutants were verified by the dideoxy method. The MHC class I–chloramphenicol

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Abbreviations: MHC, major histocompatibility complex; CAT, chloramphenicol acetyltransferase; EC cells, embryonal carcinoma cells; *tk*, thymidine kinase gene; nt, nucleotide(s).

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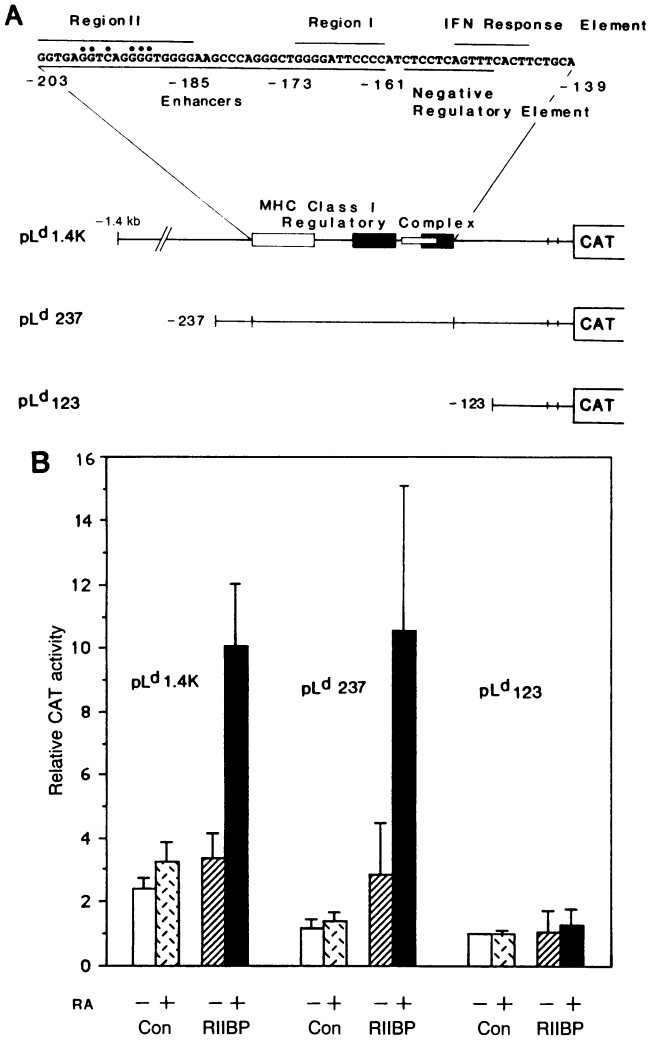

FIG. 1. (*A*) MHC class I promoter and region II. Region II (nt −203 to −184) is within the conserved MHC class I regulatory complex. G residues that contact H-2RIIBP are marked with dots. IFN, interferon. (*B*) Retinoic acid-dependent transactivation of MHC class I promoter by pRSV-H-2RIIBP. N-Tera2 cells (10^6) were transfected with pRSV-H-2RIIBP (RIIBP) or control pRSV (*Con*) and CAT reporter plasmids and were cultured with or without retinoic acid (RA, 10 μM) for 36 hr. Normalized CAT activities were expressed relative to the activity produced by pL^d123 cotransfected with the control pRSV plasmid without retinoic acid treatment (taken as 1). Values are means of three independent determinations ± SE.

acetyltransferase (CAT) reporter constructs $pL^d1.4K$, pL^d237, and pL^d123 have been described (6) (Fig. 1*A*). pRIIm-tk and pRIId-tk contained monomeric (m) and dimeric (d) region II and were generated by inserting a region II sequence (5′-GATCTATCGATAGGCGGTGAGGTCAGGGGTGGGGAA-3′ for pRIIm-tk, 5′-GATCTGGTGAGGTCAGGGGTGGGGATCGATCCGGCGGTGAGGTCAGGGGTGGGGA-3′ for pRIId-tk), with *Bgl* II ends, into the *Bam*HI site of the herpes simplex virus thymidine kinase gene (*tk*)–CAT construct pBL-CAT8+ (28).

PCR Detection of H-2RIIBP Transcripts. Total RNA was prepared from 10^7 transfected cells by the acid guanidinium thiocyanate–phenol/chloroform extraction method. Aliquots of RNA (5 μg) were treated with 1 unit of RQ1 DNase (Promega) or 10 μg of RNase A (Sigma) at 37°C for 1 hr and then heated at 100°C for 5 min. Reverse transcription was performed with 5 μg of total RNA, 10 pmol of random hexanucleotides (Pharmacia), and 200 units of Moloney murine leukemia virus reverse transcriptase (BRL) in PCR buffer (from the Perkin–Elmer/Cetus GeneAmp kit; 10× = 500 mM KCl/100 mM Tris Cl, pH 8.3/15 mM $MgCl_2$/0.1% gelatin) with 0.5 mM dNTPs at 37°C for 1 hr. Amplification of a 251-bp H-2RIIBP fragment was achieved by PCR using two specific primers (5′-CCACAGAAGTAAGGTTCCTT-3′, corresponding to the 5′ region of the simian virus 40 polyadenylylation site of pRSV2, and 5′-GCTAGCCTGAGGCCCAGATGC-3′, corresponding to the 3′ untranslated region of H-2RIIBP).

Cell Culture, Transfection, and CAT Assay. N-Tera2 D.1 cells (18), murine L fibroblasts, and monkey COS-7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, gentamicin, and glutamine. Cells (10^6) were seeded in a 100-mm culture dish 20 hr prior to transfection. Transfection was performed by the calcium phosphate precipitation method, using *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonate (Bes) buffer (29). In brief, cells were incubated with 20 μg of DNA, 10 μg of pRSV-H-2RIIBP, 5 μg of CAT reporter plasmid, and 5 μg of pCH110 (ref. 30; Pharmacia) overnight, washed, and further incubated for 36 hr in the above medium supplemented with various concentrations of all-*trans*-retinoic acid (Sigma). CAT activities were normalized for transfection efficiency by β-galactosidase activity produced by pCH110.

RESULTS

H-2RIIBP Enhances MHC Class I Promoter Activities in N-Tera2 Cells After Retinoic Acid Treatment. The role of H-2RIIBP in MHC class I promoter activities was studied by transient cotransfection into undifferentiated N-Tera2 cells of the expression plasmid pRSV-H-2RIIBP and a reporter plasmid containing the CAT gene linked to a MHC class I promoter. Initially three reporter constructs were tested (Fig. 1*A*). $pL^d1.4K$ and pL^d237 had 1.4 kb and 237 bp of the upstream regions of the $H\text{-}2L^d$ gene and contained the conserved regulatory complex from nt −203 to nt −139. This regulatory complex includes the region II enhancer, to which H-2RIIBP binds (4, 5). This complex was deleted from the truncated reporter, pL^d123 (Fig. 1*A*). These constructs have been shown to initiate CAT transcription from the correct $H\text{-}2L^d$ initiation site (7). Cells were cotransfected with pRSV-H-2RIIBP and a reporter construct in a 2:1 weight ratio and were then treated with 10 μM retinoic acid for 36 hr (Fig. 1*B*). With the control pRSV plasmid, all three reporter constructs showed low CAT activities: pL^d237 and $pL^d1.4K$ produced only slightly higher CAT activities than pL^d123 (Fig. 1*B*), in accord with the observation that MHC class I enhancers do not function fully in undifferentiated N-Tera2 cells (J.H.S. and T.N., unpublished work). A brief, 36-hr retinoic acid treatment did not affect CAT activities from any of the reporter constructs cotransfected with the control pRSV. However, cotransfection with pRSV-H-2RIIBP resulted in an ≈3-fold increase in CAT activities by $pL^d1.4K$ and pL^d237; this increase was seen only when cells were treated with retinoic acid. Without retinoic acid, levels of CAT activities with pRSV-H-2RIIBP were comparable to those with the control pRSV. In contrast, CAT activity from the shorter, pL^d123 construct was not affected by pRSV-H-2RIIBP in either the presence or the absence of retinoic acid. The enhancement of MHC class I promoter activity by H-2RIIBP was highly reproducible. These results indicate that H-2RIIBP is capable of transactivating MHC class I promoter activity in undifferentiated N-Tera2 cells, provided that cells are treated with retinoic acid. These results also indicate that this transactivation is mediated by a sequence mapped between nt −237 and nt −123.

Transactivation was detectable at concentrations >1 μM. It appeared to peak at 50–100 μM (data not shown). Cotransfection with the control pRSV did not produce a significant increase at any retinoic acid concentration tested. N-Tera2

cells have been shown to differentiate in response to retinoic acid at concentrations ranging from 0.5 to 10 μM (18), which were comparable to those observed for H-2RIIBP transactivation. Induction of various genes in N-Tera2 cells, such as Hox-2 (22), AP-2 (23), and MHC class I, has also been found to occur in response to 10 μM retinoic acid. Thus, H-2RIIBP transactivation occurs within the "physiological" concentrations of retinoic acid in N-Tera2 cells. The effect of retinol (vitamin A) was also tested, since its structure is closely related to retinoic acid. No transactivation was detected with retinol up to 10 μM (data not shown).

PCR Detection of H-2RIIBP Transcripts Following Transient Transfection. To show that H-2RIIBP transcripts were indeed produced from the expression plasmid after transfection, PCR assays were performed. Total RNA prepared from N-Tera2 cells that had been transfected with pRSV-H-2RIIBP or with control pRSV in the same conditions as above was reverse transcribed and cDNAs from plasmid-derived H-2RIIBP transcripts were specifically amplified. Amplified fragments were visualized by ethidium bromide staining (Fig. 2). Only RNA from cells transfected with pRSV-H-2RIIBP produced the expected 251-bp fragment, identical to that produced from the pRSV-H-2RIIBP plasmid DNA. Cells transfected with control pRSV did not produce this band. Because this band was eliminated by RNase treatment but not by DNase, the band was a product of H-2RIIBP transcripts rather than the plasmid DNA, which may have contaminated the RNA preparations. These data show that the H-2RIIBP expression plasmid used in this work is functional and produces specific transcripts in N-Tera2 cells, suggesting that the observed transactivation is caused by cotransfected H-2RIIBP.

H-2RIIBP Transactivates a Heterologous *tk* Promoter Driven by Region II. To study whether H-2RIIBP transactivation is attributable to region II, we tested reporter constructs in which region II was placed in front of the herpes simplex virus *tk* promoter. pRIIm-tk had a monomeric region II, whereas pRIId-tk had a dimeric region II in front of the *tk* promoter–CAT region. Results of cotransfection of these reporters and pRSV-H-2RIIBP are summarized in Fig. 3. The control *tk* promoter construct without region II (pBL-CAT8+; ref. 28) gave a low CAT activity with the control pRSV. pRSV-H-2RIIBP did not significantly change levels of CAT activity by this construct with or without retinoic acid treatment. However, significant and reproducible transactivation was observed when either pRIIm-tk or pRIId-tk was cotransfected with pRSV-H-2RIIBP. This transactivation was again seen only when cells were treated with retinoic acid for 36 hr. Thus, region II is capable of conferring H-2RIIBP transactivation on an unrelated promoter that does not have other MHC class I cis elements. These results demonstrate that region II serves as a target for H-2RIIBP. Neither an additive nor a synergistic effect was observed for the dimerized region II reporter (pRIId-tk) over the monomeric counterpart (pRIIm-tk); the basis of this has not been studied in detail. The basal CAT activity produced by pRIIm-tk and pRIId-tk was higher than that by the control *tk*–CAT construct (pBL-CAT8+) with or without pRSV-H-2RIIBP. This presumably reflects a moderate enhancer activity elicited by region II itself, consistent with previous observations (5). A small increase detected with the control *tk* promoter after pRSV-H-2RIIBP cotransfection and following retinoic acid treatment may be a result of stimulation of the *tk* promoter itself.

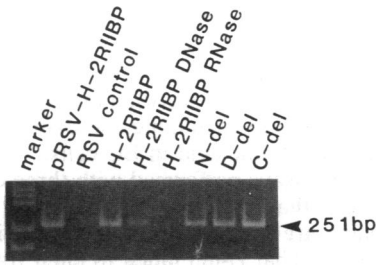

FIG. 2. PCR detection of plasmid-specific H-2RIIBP transcripts in transfected N-Tera2 cells. Total RNA (5 μg) from N-Tera2 cells transfected with pRSV-H-2RIIBP, H-2RIIBP deletion mutants, or with the control pRSV was analyzed by PCR. Primers that allow specific amplification of plasmid-derived H-2RIIBP transcripts were used. The amplified 251-bp H-2RIIBP band is indicated. ϕX174 phage DNA digested with *Hae* III was used as a size marker.

Deletion of the DNA- and the Ligand-Binding Domain, but Not of the N-Terminal Domain, Abolishes Transactivation. To assess the domains involved in H-2RIIBP transactivation, three deletion mutants of H-2RIIBP were constructed. The putative domain composition of H-2RIIBP and the position of the deletions are presented in Fig. 4*A*. N-del, D-del, and C-del had deletions of 54, 60, and 155 amino acids in the N-terminal, the DNA-binding, and the ligand-binding (C-terminal) domain, respectively. These deletions amounted to removal of more than half of each domain. These mutants also produced respective H-2RIIBP transcripts following transfection (Fig. 2). Results of cotransfection with MHC class I reporters are shown in Fig. 4*B*. While the intact pRSV-H-2RIIBP showed retinoic acid-dependent transactivation of pL^{d}1.4K, both D-del and C-del failed to elicit transactivation of this reporter. In contrast, an unexpected 2-fold enhancement in retinoic acid-dependent H-2RIIBP transactivation was noted with N-del. These results indicate that both the DNA-binding and the ligand-binding domains, but not the N-terminal domain, are required for transactivation of MHC class I promoter. The enhanced transactivation seen with N-del indicates that the N-terminal domain also has a function, perhaps an inhibitory role.

Cell-Type Restriction of H-2RIIBP Transactivation. To study whether H-2RIIBP transactivation of MHC class I promoter depends on an intracellular milieu specific for undifferentiated N-Tera2 cells, cotransfection experiments were performed with differentiated N-Tera2 cells obtained following 7 days of retinoic acid treatment. Like other EC cells, N-Tera2 cells, after differentiation, lose properties characteristic of EC cells. They grow at a reduced rate and a subpopulation of cells gives rise to neuronal cells (18).

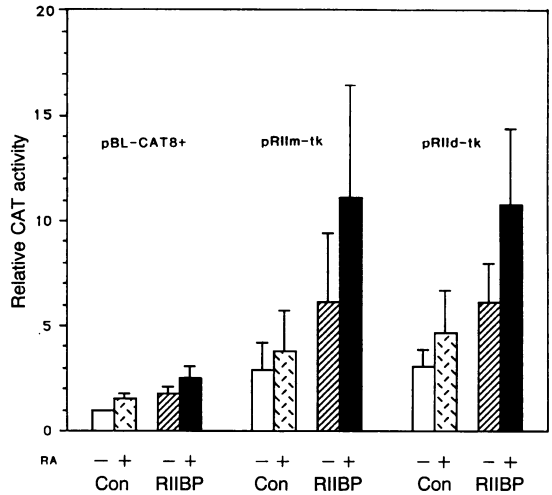

FIG. 3. Retinoic acid-dependent transactivation of region II–*tk* promoters by H-2RIIBP. pRIIm-*tk* and pRIId-*tk* were derived from the control *tk*–CAT construct pBL-CAT8+. Cells were transfected as in Fig. 1 except that the *tk*–CAT constructs were used as reporters. Values represent means of three independent determinations ± SE, expressed relative to the activity produced by pBL-CAT8+ as in Fig. 1.

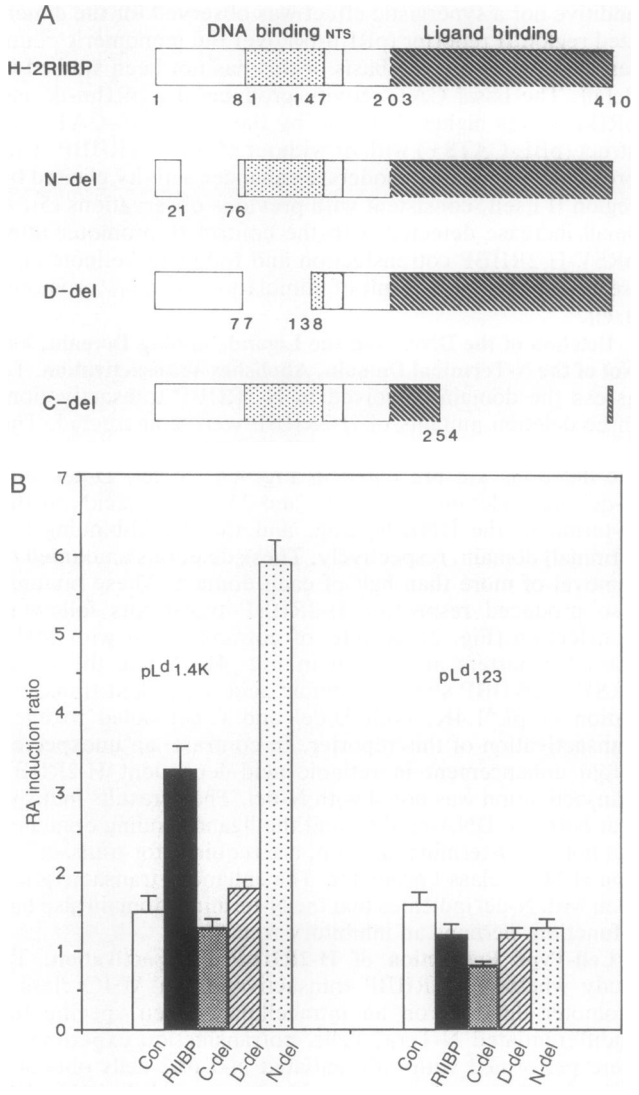

FIG. 4. Analysis of H-2RIIBP deletion mutants. (*A*) Deletion constructs. NTS, the putative nuclear translocation signal. Numbers represent amino acid positions. (*B*) Retinoic acid (RA) induction ratios. Cells were cotransfected with the above mutants and pL^{d}1.4K or pL^{d}123 as in Fig. 1. Normalized CAT activities seen with retinoic acid treatment were expressed relative to those without retinoic acid treatment. Data represent means of four determinations ± SE.

Concomitantly, the majority of cells begin to express MHC class I molecules on the cell surface. As seen in Fig. 5, differentiated N-Tera2 cells did not support H-2RIIBP transactivation of pL^{d}1.4K in either the presence or the absence of retinoic acid. In differentiated N-Tera2 cells, pL^{d}1.4K produced much greater CAT activity than pL^{d}123, whereas in undifferentiated N-Tera2 cells, pL^{d}1.4K and pL^{d}123 gave similar promoter activities (Fig. 1*B*). In addition, retinoic acid treatment slightly increased CAT activity by pL^{d}1.4K without H-2RIIBP. These results are consistent with the acquisition of MHC class I enhancer activity during retinoic acid-induced differentiation (J.H.S. and T.N., unpublished work). Further, murine L fibroblasts and monkey COS-7 cells were tested (Fig. 5 for L cells). The former express MHC class I genes at high levels and have been used for analysis of MHC class I promoter activity (11), while the latter have been used to study roles of hormone receptors (e.g., ref. 31). No significant transactivation by H-2RIIBP was observed in these cells. Likewise, a human breast tumor cell line, MCF-7, did not show transactivation (data not shown). These results indicate that H-2RIIBP transactivation

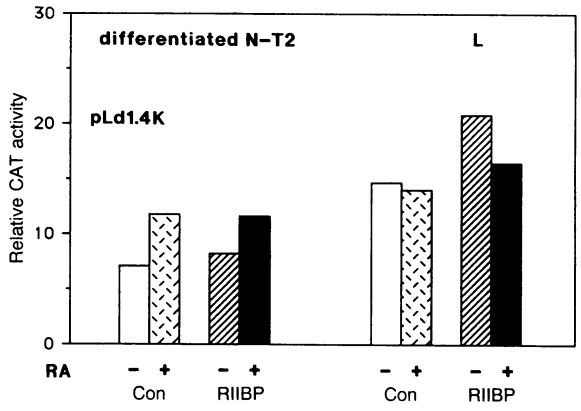

FIG. 5. Cell-type restriction of H-2RIIBP transactivation. Cells were cotransfected with H-2RIIBP and MHC class I reporters with and without retinoic acid as in Fig. 1. Normalized CAT activities produced by pL^{d}1.4K are expressed relative to the activity produced by pL^{d}123 as in Fig. 1. N-Tera2 cells treated with 10 μM retinoic acid for 7 days and L fibroblasts were used for transfection. After transfection, cells were treated with or without 10 μM retinoic acid (RA) for 36 hr, and CAT assays were performed as in Fig. 1. Values shown are the means of duplicate determinations.

requires a specific cellular environment, and that undifferentiated N-Tera2 cells, but not differentiated cells, fulfill this requirement. It seems unlikely that the lack of transactivation is due to high levels of endogenous H-2RIIBP expressed in these cells, which might obscure the effect of transactivation, because the levels of H-2RIIBP mRNA and protein in differentiated N-Tera2 cells and in L cells were low, comparable to those seen in undifferentiated cells (J.H.S. and T.N., unpublished work).

DISCUSSION

Transfection of pRSV-H-2RIIBP led to production of H-2RIIBP mRNA and to enhancement of MHC class I promoter activity in undifferentiated N-Tera2 cells. This enhancement was retinoic acid-dependent and mediated through region II, to which H-2RIIBP binds: the region II sequence alone conferred retinoic acid-dependent transactivation on a heterologous *tk* promoter. These results demonstrate that H-2RIIBP can serve as a transcription factor to enhance MHC class I promoter activity in undifferentiated N-Tera2 cells. Retinoic acid concentrations required for H-2RIIBP transactivation correlated with those capable of inducing differentiation as well as expression of MHC class I and other genes in N-Tera2 cells (22, 23). In addition, retinoic acid receptor α also transactivates MHC class I promoters with concentrations of retinoic acid similar to those required for H-2RIIBP (T.N., unpublished work). These observations lend credence to the physiological relevance of H-2RIIBP transactivation presented in this paper. Because the GG(T/A)CA motif is present in cis-acting elements of other genes, including hormone-responsive elements (B.-Z.L., unpublished work), it may be postulated that H-2RIIBP has a role in regulating the expression of other genes affected by retinoic acid in EC cells.

Cotransfection assays performed with three deletion constructs indicated that both the DNA-binding and ligand-binding domains are required for transactivation of MHC class I promoters. That D-del failed to elicit transactivation indicates that binding of H-2RIIBP to the target element is essential for transactivation, and is consistent with results (Fig. 3) that show region II to be the direct target for the transactivation. The C-del mutant also failed to elicit transactivation. Since this mutant retained the putative nuclear translocation signal, the failure of transactivation is unlikely to be due to a defect in translocation. This mutant, however,

lacked a sequence corresponding to the putative dimerization domain (32). The dimerization domain is present in some but not all nuclear hormone receptors and is composed of heptad repeats. The lack of transactivation by C-del may thus be ascribed to a loss of a function relevant to the dimerization of H-2RIIBP. Both the DNA-binding and C-terminal domains have been shown to be critical for transcription of target genes by the estrogen (33), thyroid hormone (34), and glucocorticoid (35, 36) receptors. However, it is possible that the lack of transactivation by these mutants is due to gross alterations of H-2RIIBP tertiary structure created by the deletions rather than the loss of specific functions assigned to distinct domains. Removal of the bulk of the N-terminal domain did not abrogate H-2RIIBP transactivation. Similar results have been noted for some but not all receptors (33, 34, 36, 37). It is likely that the N-terminal domain is involved in functions not studied in this work, as judged by the elevated transactivation by N-del. One of the most notable aspects of H-2RIIBP transactivation is its cell-type restriction. Among various cells tested, only undifferentiated N-Tera2 cells demonstrated H-2RIIBP transactivation. It is significant that N-Tera2 cells, after they had differentiated, no longer supported H-2RIIBP transactivation, indicating that the observed cell-type restriction reflects developmental specificity of H-2RIIBP function. These observations may be due to such possibilities as (*i*) the presence of an inhibitory factor in cells other than undifferentiated N-Tera2 cells that overrides H-2RIIBP transactivation or (*ii*) domination of other cis elements in other cells that obscures a transactivating effect of H-2RIIBP. However, the simplest interpretation is that H-2RIIBP does not function alone but acts together with an additional factor that is expressed in a cell type-specific fashion. This idea is consistent with reports that some nuclear hormone receptors form a heterodimer (38, 39). Glass *et al.* (40) reported that the retinoic acid receptor α heterodimerizes not only with the thyroid hormone receptor α but with multiple hitherto unidentified cellular factors that are expressed in a cell type-specific fashion. Requirement of unidentified cellular factors for binding and function of the thyroid hormone receptors has also been reported by Lazar and Berrodin (41) and Murray and Towle (42). A recent report by Rottman *et al.* (43) indicates that RXRα heterodimerizes with an unknown cellular protein(s) and enhances the apolipoprotein A-I gene promoter. The capacity to form a heterodimer may be limited to certain members of the superfamily, as some receptors are reported to function as a homodimer (44–46). That the C-del mutant, devoid of most of the dimerization domain, failed to achieve transactivation is consistent with the involvement of a heterodimerizing cofactor. Further supporting this idea, H-2RIIBP is capable of dimerizing with other members of the superfamily (B.-Z.L., unpublished work). The relatively weak transactivation of CAT expression observed in this study might have been caused by a limited amount of a cofactor present in N-Tera2 cells. Based on this reasoning, it is possible that the retinoic acid requirement observed in this work represents binding of retinoic acid to the postulated cofactor(s) rather than to H-2RIIBP itself. Finally, the present work shows that H-2RIIBP is involved in retinoic acid-mediated developmental regulation of MHC class I gene expression. Further investigations into the postulated cofactor may help elucidate the basis of the broad regulatory capacity of H-2RIIBP.

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